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Microidium phyllanthi-reticulati sp. nov. on *Phyllanthus reticulatus*

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Abstract—A new powdery mildew species of the genus *Microidium* (*Erysiphaceae*) found on *Phyllanthus reticulatus* (*Phyllanthaceae*) in Thailand is described. *Microidium phyllanthi-reticulati* [= *Oidium phyllanthi* var. *reticulati*] is characterized by having amphigenous colonies on the host surface, long conidiophore foot-cells twisted at the base, and barrel-shaped to cylindrical conidia with *Microidium*-type germ tubes. The new species differs from the allied and morphologically very similar *M. phyllanthi* in producing smaller conidia and conidiophores with longer foot-cells. Analyses of rDNA ITS + 28S sequence data support the separation of *M. phyllanthi-reticulati* from *M. phyllanthi*. The new species is described and illustrated in detail, and compared with other species assigned to *Microidium*.

Key words—Asia, *Erysiphales*, phylogeny, *Euphorbiaceae*

Introduction

The name *Oidium phyllanthi* J.M. Yen was originally proposed for a powdery mildew found on *Phyllanthus urinaria* in Taiwan; two types of conidia and conidiophores were described—primary conidiophores with cylindrical to oval conidia and secondary conidiophores producing with cylindrical conidia (Yen 1967). Subsequently *O. phyllanthi* has also been recorded on *Phyllanthus acidus*, *P. amarus*, *P. niruri*, *P. reticulatus*, and *P. rheedei* (Braun & Cook 2012, as *Microidium phyllanthi*). Narayanswami & Ramakrishnan (1969) published an illegitimate later homonym, *O. phyllanthi* Narayanas. & K. Ramakr., based on an Indian collection on *Phyllanthus niruri* var. *amarus* [≡ *P. amarus*], but provided only a meagre description confined to the size of conidia (14.5–20

× 2.2–9.1 µm); Hosagoudar (1991) introduced *O. ramakrishnanii* Hosag. as a legitimate replacement name for this species.

Oidium subg. *Microidium* To-anun & S. Takam. was introduced by To-anun et al. (2005) based on molecular phylogenetic analyses of the rDNA of 18S and 28S regions and a morphological re-assessment of specimens of *Oidium phyllanthi* found on *Phyllanthus acidus*, *P. amarus*, and *P. reticulatus*. The specimens concerned were unique due to the formation of a different type of conidial germination, designated as *Microidium*-type (To-anun et al. 2002). Subsequent molecular analysis using a 18S and 28S rDNA (D1 and D2 regions) combined dataset indicated that the sequences from *O. phyllanthi* on *P. acidus*, *P. amarus*, and *P. reticulatus* represented a unique lineage within the *Erysiphaceae* and that the sequence of a *P. reticulatus* isolate differed from the *P. acidus* and *P. amarus* isolates by 1.38% in the 28S rDNA D1/D2 sequence (To-anun et al. 2005).

Braun & Cook (2012: 624) later raised *Oidium* subg. *Microidium* to generic rank as *Microidium* (To-anun & S. Takam.) To-anun & S. Takam. Here we present ITS + 28S analyses of *Microidium* on *Phyllanthus*, including new sequences from 15 specimens and additional sequences retrieved from GenBank, together with detailed morphological examinations of all of these specimens.

Materials & methods

Morphological examination

Specimens were collected in northern Thailand between November 2004 and January 2016. The host name, collection data, localities, and the name of the collector were noted. Morphological examinations were carried out as outlined in Meeboon & Takamatsu (2015a). Hyphae, conidiophores, and conidia were stripped from the leaf surfaces with a clear adhesive tape, mounted on a microscope slide with the fungal mycelium upper most, and examined in water using a light microscopy with phase contrast at 20×, 40×, and 100× (oil immersion). Thirty conidia were measured from each specimen examined.

Conidial germ tubes were examined using the method of Hirata (1942). The inner surface cell layer of onion scales was cut with a razor in a size of 1 cm^2 and stripped off by a clean forceps. The cell layer was kept in 80% ethanol more than two weeks and rinsed with tap water for 30 min before use. The cell layer was put on a microscope slide, followed by removing excess water with filter paper, and powdered with the conidia. The conidia-covered cell layer, floating on distilled water in a Petri dish, was incubated at 20–25°C for 24h until microscopic examination.

Specimens were deposited in the herbarium of the National Museum of Nature and Science, Tsukuba, Japan (TNS), and Mie University Mycological Herbarium, Tsu, Japan (MUMH).

Phylogenetic analysis

Whole-cell DNA was extracted by the Chelex method (Walsh et al. 1991, Hirata & Takamatsu 1996). The nucleotide sequences of the 5′-end of the 28S rRNA gene (including domains D1 and D2) were determined in accordance with Meeboon & Takamatsu (2015b). The rDNA internal transcribed spacer (ITS) region including 5.8S rDNA was amplified using primers ITS5 (White et al. 1990) and p3 (Kusaba & Tsuge 1995) for the first amplification. One microlitre (µl) of the first reaction mixture was used for the second amplification with the partial nested primer sets ITS5 and ITS4 (White et al. 1990). The ITS5/ITS4 fragment was subjected to sequencing using ITS1, ITS4, T3, and T4 (Hirata & Takamatsu 1996). Representative sequences gained in this study were deposited in DNA databases (DDBJ, EMBL, GenBank). These sequences were aligned with the 28S rDNA D1/D2 sequence in To-anun et al. 2005 using MUSCLE (Edgar 2004) implemented in MEGA version 6 (Tamura et al. 2013). Alignments were further manually refined using MEGA and deposited in TreeBASE (http://www.treebase.org/) under the accession number S12714. Phylogenetic trees were obtained from the datasets by using the maximum parsimony (MP) method implemented in PAUP* 4.0b10 (Swofford 2002) with heuristic search option using 'tree bisection-reconstruction' (TBR) algorithm with 100 random sequence additions to find global optimum tree. All sites were treated as unordered and unweighted, with gaps treated as missing data. The strength of internal branches of the resulting trees was tested with bootstrap (BS) analyses (Felsenstein 1985) using 1000 replications with step-wise addition option set as simple. BS values 70% or higher were given. Tree scores, including tree length, consistency index (CI), retention index (RI), and rescaled consistency index (RC) were also calculated.

Taxonomy

Microidium phyllanthi-reticulati Meeboon & S. Takam., **sp. nov.** FIG. 1

MYCOBANK MB 800332

= *Oidium phyllanthi* var. *reticulati* N. Ahmad, A.K. Sarbhoy, Kamal & D.K. Agarwal, Indian Phytopathol. 57: 479. 2004 [as "*reticulatus*"].

Differs from *Microidium phyllanthi* by its smaller conidia and its longer foot-cells.

TYPE—Thailand, Chiang Mai province, Chiang Mai University, on living leaves of *Phyllanthus reticulatus* Poir. (*Phyllanthaceae*), 22 December 2004, J. Meeboon (**Holotype**, TNS-F-46801; **isotype**, MUMH 3364; GenBank AB719952, LC259489).

ETYMOLOGY—Named after the host species, *Phyllanthus reticulatus*.

COLONIES amphigenous, mycelium white, effuse, confluent, forming irregular white patches or covering the whole leaf surface (also on young stems); hyphae hyaline, branched, septate, 3–5 µm diam., thin-walled, smooth. APPRESSORIA well developed, lobed to multilobed, opposite in pairs or single. CONIDIOPHORES formed singly on hyphal cells, arising ± centrally from the upper cell surface, 82–183 × 4–8 µm, foot-cells long, cylindrical, twisted at the base, 40–118 ×

4–8 µm, with a basal septum near the junction with the supporting hypha or elevated up to 5 µm, conidiophores producing 3–6 conidia in chains (catenescent). CONIDIA cylindrical to doliiform cylindrical, (7–)15–20(–23) × (7–)8–10(–13) µm, with a large length/width ratio of 1.8–2.5, containing oil-drop-like inclusions but devoid of conspicuous fibrosin bodies, producing perihilar germ tubes ("on shoulders"), broadly club-shaped, terminating in nipple-shaped or lobed appressoria (*Microidium*-type).

ADDITIONAL SPECIMENS EXAMINED—THAILAND, CHIANG MAI, 27 December 2002, J. Meeboon (MUMH 3363; GenBank AB719953); 5 January 2007, J. Meeboon (MUMH 3824; GenBank AB719954). CHIANG RAI, 12 January 2008, J. Meeboon (MUMH 3823; GenBank AB719955); NAN, 3 February 2002, S. Kom-un (MUMH 1761; GenBank LC259486, AB120758).

COMPARATIVE SPECIMENS EXAMINED—*Microidium phyllanthi*: THAILAND, CHIANG RAI, 8 March 2004, J. Meeboon (MUMH 3361; GenBank AB719943); 8 March 2004, J. Meeboon (MUMH 3360; GenBank AB719944); 2 November 2013, J. Meeboon (MUMH 5743; GenBank LC259492); 18 December 2015, J. Meeboon (MUMH 6624); 26 December 2016, J. Meeboon (MUMH 6880, GenBank LC259488); 3 January 2016, J. Meeboon (MUMH 6668; GenBank LC169073, LC259490); 15 December 2014, J. Meeboon (MUMH 6676; GenBank LC169074, LC259491); 3 November 2013, J. Meeboon (MUMH 5744; GenBank LC259493). MAE HONG SON, 26 November 2003, J. Meeboon (MUMH 3366; GenBank AB719945). CHIANG MAI, 13 February 2002, J. Meeboon (MUMH 1492; GenBank AB719946); 13 February 2002, J. Meeboon (MUMH 3362; GenBank AB719947); 25 February 2004, J. Meeboon (MUMH 3357; GenBank AB719948); 25 February 2004, J. Meeboon (MUMH 3822; GenBank AB719949); 3 February 2007, J. Meeboon (MUMH 3352; GenBank AB719950); 3 February 2007, J. Meeboon (MUMH 1782; GenBank LC259487, AB120755). NAN, 3 February 2002, S. Kom-un (MUMH 1778; GenBank AB719951, AB120754).

Phylogenetic analysis

The combination of ITS + 28S sequences from 20 specimens of *Microidium* from *Phyllanthus* (including the three specimens included in the analyses of To-anun et al. 2005: MUMH 1761, 1778, and 1782) were aligned and analysed with *Neoerysiphe galeopsidis* (DC.) U. Braun (MUMH 630) as outgroup. The alignments were carefully edited manually, with ambiguously aligned sites deleted from further analysis. Of the remaining 1239 total characters, 1036 were constant, 108 were variable but parsimony uninformative, and 95 were parsimony-informative. The MP analysis produced about 200K equally parsimonious trees with 211 steps. Topologies were almost consistent among the trees except for branching orders of the terminal branches and branch length; a typical tree is shown in FIG. 2. The sequences obtained from the 15 specimens of *M. phyllanthi* from *P. acidus* and *P. amarus* were identical. The sequences from the five specimens from *P. reticulatus* formed a separate clade

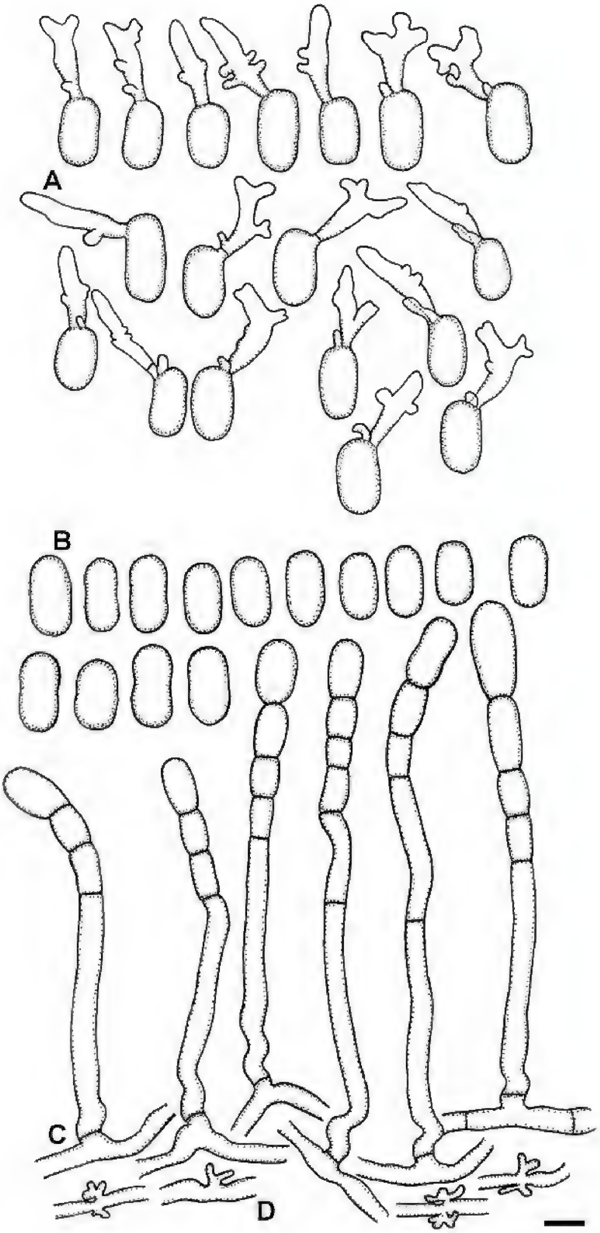

FIG. 1. *Microidium phyllanthi-reticulati* on *Phyllanthus reticulatus* (holotype, TNS-F-46801): A. Conidia with germ tubes; B. Conidia; C. Conidiophores; D. Appressoria. Scale bar = 10 µm.

with 100% bootstrap support; they differed from the sequences from the *P. acidus* and *P. amarus* specimens by 13.2% (ITS) and 4.42% (28S).

Discussion

Braun & Cook (2012) recognized three species currently assigned to *Microidium*, *M. phyllanthi* (J.M. Yen) To-anun & S. Takam., *M. bauhiniicola* (U. Braun & Dianese) U. Braun & Dianese, and *M. agatidis* (É.E. Foëx) U. Braun. *Microidium phyllanthi* is distinguished from *M. agatidis* and *M. bauhiniicola* by conidiophores with foot-cells that are twisted at the base (Braun & Cook 2012). The foot-cells of *M. agatidis* and *M. bauhiniicola* are straight, and both species are separated based on the sizes of their foot-cells, hosts, and differences in the geographical distribution: *M. agatidis*, with 15–35 µm long foot-cells, was found on *Sesbania grandiflora* in Asia, whereas *M. bauhiniicola*, with 30–90 µm long foot-cells, was found on *Bauhinia* spp. in South America (Braun & Cook 2012).

Ahmad et al. (2004) described a new variety, *O. phyllanthi* var. *reticulati,* based on the size of the conidia—slightly smaller than in *O. phyllanthi* J.M. Yen [≡ *Microidium phyllanthi*] and slightly larger than in the illegitimate *O. phyllanthi* Narayanas. & K. Ramakr. [≡ *Oidium ramakrishnanii*]. Ahmad et al. (2004) also emphasized that these three entities should be considered different varieties of *O. phyllanthi*. The relation between *Microidium phyllanthi* s. str., described from Taiwan on *P. urinaria*, and collections on *P. acidus* and *P. amarus* (available name: *Oidium ramakrishnanii*) currently cannot be clarified due to lack of sequence data from Taiwanese or other *Microidium* collections on *P. urinaria*.

The powdery mildew specimens found on *P. reticulatus* in Thailand are morphologically very similar to the original description of *O. phyllanthi* var. *reticulati* [conidia (7–)15–20(–23) × (7–)8–10(–13) µm vs. 12.7–26.7 × 6–8 µm in *O. phyllanthi* var. *reticulati*] found on the same host species. The colonies in the specimens found in Thailand grow on both sides of the leaf surface (amphigenous), whereas in *O. phyllanthi* var. *reticulati* they were described as often formed epiphyllously. Ahmad et al. (2004) did not describe the conidial germination.

Compared to the *Microidium* species keyed out in Braun & Cook (2012), the new species is morphologically close to *M. phyllanthi* based on having conidiophore foot-cells twisted at the base. However, *M. phyllanthi-reticulati* differs from *M. phyllanthi*, which has larger conidia (20–30(–40) × 7–11.5 µm) and shorter foot-cells 20–105 µm (Braun & Cook 2012). *Microidium phyllanthi*

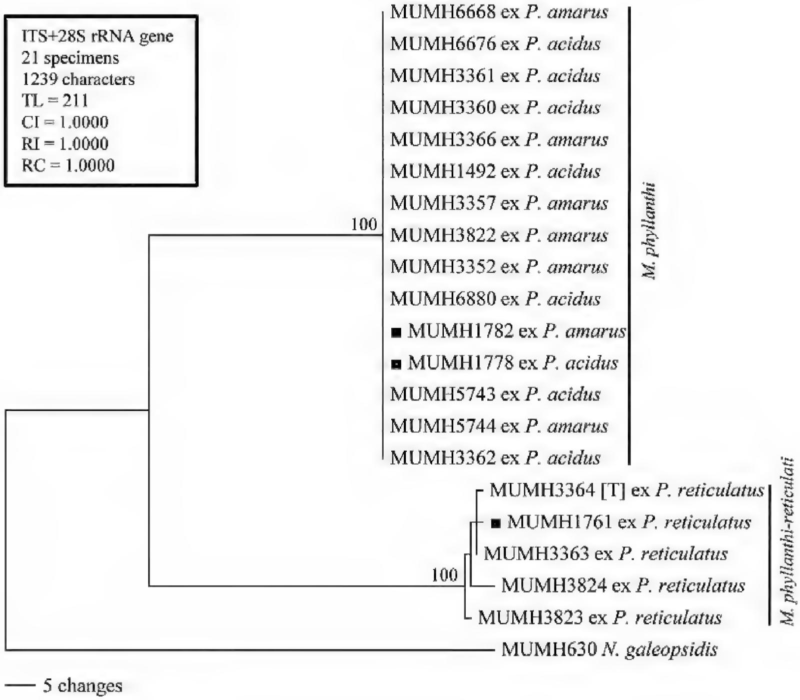

FIG. 2. Phylogenetic analysis of the nucleotide sequences of ITS + 28S regions for 21 specimens of *Microidium* from *Phyllanthus*; the three specimens included in the analyses of To-anun et al. (2005) are marked with '■'. The isotype specimen of *M. phyllanthi-reticulati* is indicated with '[T]'. The tree is one of 200K equally parsimonious trees with 211 steps, which was obtained by the parsimony method. Gaps were treated as missing data. Horizontal branch lengths are proportional to the number of nucleotide substitutions that were inferred to have occurred along a particular branch of the tree. Percentage bootstrap support ≥50% (1000 replications) is shown on branches.

is widespread in Africa (Ghana, Mauritius) and Asia (India, Indonesia, Sri Lanka, Taiwan, Thailand), whereas *M. phyllanthi-reticulati* is known only from India and Thailand. Furthermore, the phylogenetic analyses of the ITS + 28S regions of the *Phyllanthus* powdery mildews examined support segregation of *M. phyllanthi-reticulati* on *P. reticulatus* from *M. phyllanthi* on *P. acidus* and *P. amarus* (FIG. 2).

We refrain from using the name *O. phyllanthi* var. *reticulati* as basionym for a new combination at species level since neither the varietal type material nor any other Indian specimens were available for morphological and molecular examinations. Additionally, the protologue cited an incorrect orthographic variant of the varietal epithet ("*reticulatus*"), and other incorrect variants ("*reticulatum*" and "*reticulatae*") have been listed in databases and in the literature; the correct epithet orthography is "*reticulati*" (Latin genitive case of the host epithet "*reticulatus*"). Nomenclaturally, our introduction of a new species name is legitimate, since varietal names do not have priority at species level (McNeill et al. 2012; Art. 11.2).

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